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Structural and functional analysis of bacterial DNA polymerase X involved in DNA repair processes

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DNA polymerases are generally classified into seven families, the A, B, C, D, X, Y and reverse transcriptase (RT) ones. In these families, the X-family DNA polymerases (PolXs) comprise a highly conserved DNA polymerase family found in all kingdoms. Mammalian PolXs are further classified into six members: DNA polymerase beta (Polbeta), lambda (Pollambda), mu (Polmu), sigma1 (Polsigma1) and sigma2 (Polsigma2) and terminal deoxynucleotidyl transferase (TdT). These mammalian PolXs are involved in DNA repair and other DNA-processing pathways, but the cellular functions of bacterial PolXs are less known. Many bacterial PolXs have a polymerase and histidinol phosphatase (PHP) domain at their C-termini in addition to a PolX core (POLXc) domain (Figure 1), and possess 3'-5' exonuclease activity [1]. Although both domains are highly conserved in bacteria, structural arrangement of POLXc and PHP domains and the activities of each domain are little known. To reveal molecular functions and structural information of bacterial PolX, we determined the crystal structure of *Thermus thermophilus* HB8 PolX (ttPolX) at 1.4 Å resolution. Overall arrangement of the POLXc and PHP domains of ttPolX was similar to that of another bacterial PolX, however, subdomains of the POLXc domain adopted a novel arrangement. The active-site structure of the PHP domain was similar to that of other phosphoesterase enzymes. This similarity suggests that the PHP domain has a phosphoesterase activity. Furthermore, we measured *Kd* values for binding of nucleotides to ttPolX by surface plasmon resonance and isothermal titration calorimetry to evaluate physiological state of ttPolX. Our findings provide a molecular insight into the functional domain organization and the structural mechanism of bacterial PolXs.

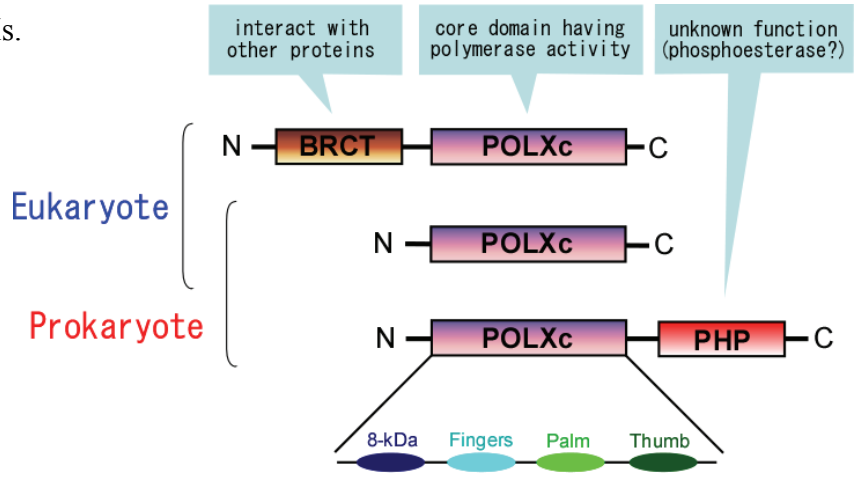

Figure 1. Domain organization of PolXs.

Reference

[1] Nakane *et al.* (2009) *Nucleic Acids Res.* **37**(6), 2037-2052